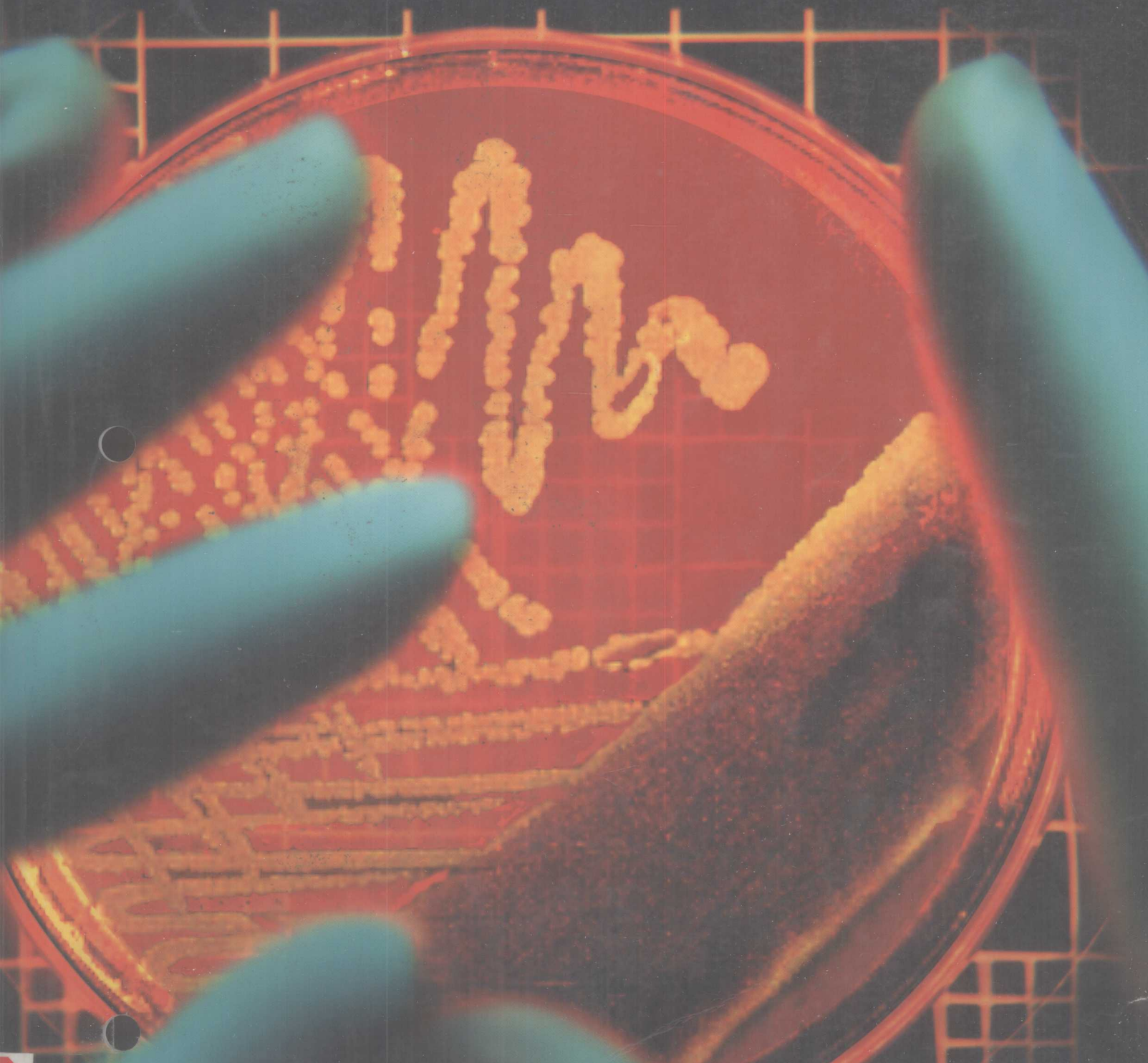
MICROBIOLOGY

A LABORATORY MANUAL SEVENTH EDITION



CAPPUCCINO • SHERMAN

Microbiology

A LABORATORY MANUAL

SEVENTH EDITION

James G. Cappuccino

Natalie Sherman

State University of New York

Rockland Community College

Benjamin Cummings

San Francisco Boston New York

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DEDICATION

It is with great pride that I dedicate this book to the memory of Natalie Sherman. She was my friend, colleague, and coauthor for 32 years. Her passion for teaching was only exceeded by her ability to teach her students well. They have become the beneficiaries of her unique talent.

Microbiology is a dynamic science. It is constantly evolving as more information is added to the continuum of knowledge, and as microbiological techniques are rapidly modified and refined. The seventh edition of *Microbiology: A Laboratory Manual* continues to provide a blend of traditional methodologies with more contemporary procedures to meet the pedagogical needs of all students studying microbiology. As in previous editions, this seventh edition contains a large number of diverse experimental procedures, providing instructors with the flexibility to design a course syllabus that meets their particular instructional approach. For this edition, I have focused on updating the terminology, equipment, and procedural techniques used in the experiments. I also modified and clarified the background information and experimental procedures and revised the color-plate insert. These changes will further facilitate student understanding and performance of microbiological procedures.

The structure of the manual remains essentially the same as in the earlier editions. Comprehensive introductory material is given at the beginning of each major area of study, and specific explanations and detailed directions precede each experiment. This approach augments, enhances, and reinforces course lectures, thereby enabling students to comprehend more readily the concepts and purposes of each experiment. This will be a further asset to those in institutions in which the laboratory and lecture sections are not taught concurrently. Finally, this manual should reduce the time required for explanations at the beginning of each laboratory session and thus make more time available for performing the experiments.

The wide variety of experiments was critically selected and tested to facilitate effective instruction in the basic principles and techniques in a variety of microbiological areas. Thus, this laboratory manual provides a wide spectrum of exercises suitable for use in elementary and advanced general microbiology courses, as well as in allied health programs. Also, the procedures have been carefully designed so that the supplies, equipment, and instrumentation commonly found in undergraduate institutions will suffice for their successful execution.

The manual consists of 79 exercises arranged in 15 parts. The exercises progress from those that are basic and introductory, requiring minimal manipulations, to those that are more complex, requiring more sophisticated skills.

I have created two new experiments for the seventh edition. In Part II, I added an experiment on darkfield microscopy, providing a hands-on introduction to this important means of microbial observation. In Part XIII, I included a bacterial transformation experiment, which addresses the modern biotechnology of DNA transformation and the history of scientific experimentation that has led to our current understanding of it.

Part I, on **basic laboratory techniques for isolation, cultivation, and cultural characterization of microorganisms,** introduces basic procedures used for isolation and cultivation of microorganisms.

Part II, on **microscopy,** introduces the use and care of the microscope for the study of microorganisms.

Part III, on **bacterial staining,** focuses on procedures for bacterial smear preparation, visualization, and differentiation of microorganisms and cell structures.

Part IV focuses on **cultivation of microorganisms, nutritional and physical requirements, and enumeration of microbial populations.**

Part V, on **biochemical activities,** introduces the varied cellular enzymatic activities that may be used for differentiation and identification of specific groups of microorganisms.

Parts VI, VII, and VIII introduce the areas of **protozoology, mycology,** and **virology.**

Part IX, **control of microbial growth,** discusses the antimicrobial activities of various physical and chemical agents.

Parts X and XI are concerned with the sanitary aspects of **food** and **water,** as well as the fermentative role of microorganisms in the production of some beverages and food products.

Part XII, on the **microbiology of soil,** discusses the role of soil microorganisms in the nitrogen cycle and antibiotic production.

Part XIII, on **bacterial genetics,** presents selected experiments to illustrate genetic principles using bacterial systems.

Parts XIV and XV, on **medical microbiology** and **immunology,** highlight both the conventional and the more recent rapid clinical screening methodologies used for the isolation and identification of pathogenic microorganisms. To circumvent the high cost associated with some of the newer experimental methodologies, it is suggested that these procedures be performed as demonstrations.

The format of each exercise is intended to facilitate presentation of the material by the instructor and to maximize the learning experience. To this end, each experiment is designed as follows:

Purpose: Defines the specific principles and/or techniques to be mastered.

Principle: An in-depth discussion of the microbiological concept or technique and the specific experimental procedure.

Materials: To facilitate the preparation of all laboratory sessions, a list of the following materials appears under this heading:

Cultures: These are the selected test organisms that have been chosen to demonstrate effectively the experimental principle or technique under study, as well as their ease of cultivation and maintenance in stock culture. A complete listing of the experimental cultures and prepared slides is presented in Appendix 6.

Media: These are the specific media and their quantities per designated student group. Appendix 3 lists the composition and method of preparation of all the media used in this manual.

Reagents: These include biological stains as well as test reagents. The chemical composition and preparation of the reagents are presented in Appendices 4 and 5.

Equipment: Listed under this heading are the supplies and instrumentation that are needed during the laboratory session. The suggested equipment was selected to minimize expense while reflecting current laboratory technique.

Procedure: Explicit instructions augmented by diagrams aid in the execution and interpretation of the experiments.

Observations and Results: Tear-out sheets located at the end of each exercise facilitate interpretation of data and subsequent review by the instructor.

Review Questions: Questions on tear-out report sheets aid the instructor in determining the student's ability to understand the experimental concepts and techniques. Questions that call for more critical thinking are indicated by the symbol shown to the left.

A caution icon has been placed at the beginning of experiments utilizing procedures that may use **potentially pathogenic materials.** The instructor may wish to perform some of these experiments as demonstrations.

Safety precautions that should be followed during procedures appear throughout the manual. In this edition they have been highlighted as boxes.

I hope that this manual will serve as a vehicle for the development of manipulative skills and techniques essential for understanding the integrated complexity of the biochemical structure and function of the single cell. This will enable an extension of these principles toward a better understanding of the more complex, higher forms of life. Ultimately, I hope that some students might further pursue the study of life at the molecular level or apply these laboratory skills in the vocational fields of applied microbiology and allied health.

Instructor Resources

The *Instructor's Guide* (0-8053-2837-8) has been completely updated for the Seventh Edition, and contains a new Tips section and added tables of media and equipment. The new Instructor's CD-ROM, just added for this edition provides adopters with over 300 photographs of microbiological culture slides, plates, and test tubes.

Acknowledgments

I wish to express my sincere gratitude to the following instructors for their reviews of the sixth edition and/or the two new experiments I have added. Their comments and direction contributed greatly to the seventh edition.

Bernard Arulanandam, University of Texas at San Antonio; Sheila Brady-Root, Nazareth College; Beverly J. Brown, Nazareth College; John Chikwem, Lincoln University; Michael A. Davis, Central Connecticut State University; Ernest M. Hannig, University of Texas at Dallas; Kirkwood M. Land, City College of San Francisco; Sue Looney, University of Alaska Anchorage; S. Jane A. Molinaro, Immaculata University; Tim Mullican, Dakota Wesleyan University; Charles B. Pumpuni, Northern Virginia Community College; Terrill Smith, City College of San Francisco; Curt W. Spanis, University of San Diego; Amy Treonis, Creighton University.

The new edition has also benefited from the contribution of Dr. David B. Alexander at the University of Portland, who provided us with permission to use his fine photos in the color insert.

Likewise, I wish to extend my appreciation to the staff at Benjamin Cummings, whose expertise and technical skills have guided Natalie and me over the many years. David Novak, Production Editor, and Marie Beaugureau, Associate Editor, provided invaluable direction in this new edition; their dedication to the highest standards has left its mark on every page of the manual. Last, but certainly not least, I wish to express my gratitude to the microbiology laboratory technicians at Rockland Community College—Ms. Joan Grace, who early on performed all the experiments to ensure their success when repeated by the students, and Ms. Roz Wehrman, who is presently following in Joan's footsteps.

James G. Cappuccino

General Rules and Regulations

A rewarding laboratory experience demands strict adherence to prescribed rules for personal and environmental safety. The former reflects concern for your personal safety in terms of avoiding laboratory accidents. The latter requires that you maintain a scrupulously clean laboratory setting to prevent contamination of experimental procedures by microorganisms from exogenous sources.

Because most microbiological laboratory procedures require the use of living organisms, an integral part of all laboratory sessions is the use of aseptic techniques. Although the virulence of microorganisms used in the academic laboratory environment has been greatly diminished because of their long-term maintenance on artificial media, *all microorganisms should be treated as potential pathogens* (organisms capable of producing disease). Thus, microbiology students must develop aseptic techniques (free of contaminating organisms) in the preparation of pure cultures that are essential in the industrial and clinical marketplaces.

The following basic steps should be observed at all times to reduce the ever-present microbial flora of the laboratory environment.

1. Upon entering the laboratory, place coats, books, and other paraphernalia in specified locations—never on bench tops.
2. Keep doors and windows closed during the laboratory session to prevent contamination from air currents.
3. At the beginning and termination of each laboratory session, wipe bench tops with a disinfectant solution provided by the instructor.
4. Do not place contaminated instruments, such as inoculating loops, needles, and pipettes, on bench tops. Loops and needles should be sterilized by incineration, and pipettes should be disposed of in designated receptacles.
5. On completion of the laboratory session, place all cultures and materials in the disposal area as designated by the instructor.
6. Rapid and efficient manipulation of fungal cultures is required to prevent the dissemination of their reproductive spores in the laboratory environment.

To prevent accidental injury and infection of yourself and others, observe the following regulations at all times:

1. Wash your hands with liquid detergent, rinse with 95% ethyl alcohol, and dry them with paper towels upon entering and prior to leaving the laboratory.
2. Wear a paper cap or tie back long hair to minimize its exposure to open flames.
3. Wear a laboratory coat or apron while working in the laboratory to protect clothing from contamination or accidental discoloration by staining solutions.
4. Wear closed shoes at all times in the laboratory setting.
5. Never apply cosmetics or insert contact lenses in the laboratory.
6. Do not smoke, eat, or drink in the laboratory. These activities are absolutely prohibited.
7. Carry cultures in a test tube rack when moving around the laboratory. Likewise, keep cultures in a test tube rack on the bench tops when not in use. This serves a dual purpose: to prevent accidents and to avoid contamination of yourself and the environment.
8. Never remove media, equipment, or especially, *bacterial cultures* from the laboratory. Doing so is absolutely prohibited.
9. Immediately cover spilled cultures or broken culture tubes with paper towels and then saturate them with disinfectant solution. After 15 minutes of reaction time,

remove the towels and dispose of them in a manner indicated by the instructor.

10. Report accidental cuts or burns to the instructor immediately.
11. Never pipette by mouth any broth cultures or chemical reagents. Doing so is strictly prohibited. Pipetting is to be carried out with the aid of a mechanical pipetting device only.
12. Do not lick labels. Use only self-stick labels for the identification of experimental cultures.
13. Speak quietly and avoid unnecessary movement around the laboratory to prevent distractions that may cause accidents.
14. Always wear gloves when irradiating cultures.

The following specific precautions must be observed when handling body fluids of unknown origin due to the possible imminent transmission of the HIV and hepatitis B viruses in these test specimens.

1. Wear disposable gloves during the manipulation of test materials such as blood, serum, and other body fluids.
2. Immediately wash hands if contact with any of these fluids occurs and also upon removal of the gloves.
3. Wear masks, safety goggles, and laboratory coats if an aerosol might be formed or splattering of these fluids is likely to occur.
4. Decontaminate spilled body fluids with a 1:10 dilution of household bleach, covered with paper toweling, and allowed to react for 10 minutes before removal.
5. Place test specimens and supplies in contact with these fluids into a container of disinfectant prior to autoclaving.

I have read the above laboratory safety rules and regulations and agree to abide by them.

Name _____ Date _____

Student Preparation for Laboratory Sessions

The efficient performance of laboratory exercises mandates that you attend each session fully prepared to execute the required procedures. Read the assigned experimental protocols to effectively plan and organize the related activities. This will allow you to maximize use of laboratory time.

Preparation of Experimental Materials

Microscope Slides: Meticulously clean slides are essential for microscopic work. Commercially precleaned slides should be used for each microscopic slide preparation. However, wipe these slides with dry lens paper to remove dust and finger marks prior to their use. With a glassware marking pencil, label one end of each slide with the abbreviated name of the organism to be viewed.

Labeling of Culture Vessels: Generally, microbiological experiments require the use of a number of different test organisms and a variety of culture media. To ensure the successful completion of experiments, organize all experimental cultures and sterile media at the start of each experiment. Label culture vessels with non-water-soluble glassware markers and/or self-stick labels prior to their inoculation. The labeling on each of the experimental vessels should include the name of the test organism, the name of the medium, the dilution of sample (if any), your name or initials, and the date. *Place labeling directly below the cap of the culture tube.* When labeling Petri dish cultures, only the name of the organism(s) should be written on the bottom of the plate, close to its periphery, to prevent obscuring observation of the results. The additional information for the identification of the culture should be written on the cover of the Petri dish.

Inoculation Procedures

Aseptic techniques for the transfer or isolation of microorganisms, using the necessary transfer instruments, are described fully in the experiments in Part I of the manual. Technical skill will be acquired through repetitive practice.

Inoculating Loops and Needles: It is imperative that you incinerate the entire wire to ensure absolute sterilization. The shaft should also be briefly passed through the flame to remove any dust or possible contaminants. To avoid killing the cells and splattering the culture, cool the inoculating wire by tapping the inner surface of the culture tube or the Petri dish cover prior to obtaining the inoculum, or touch the edge of the medium in the plate.

When performing an aseptic transfer of microorganisms, a minute amount of inoculum is required. If an agar culture is used, touch only a single area of growth with the inoculating wire to obtain the inoculum. *Never drag the loop or needle over the entire surface, and take care not to dig into the solid medium.* If a broth medium is used, first tap the bottom of the tube against the palm of your hand to suspend the microorganisms. *Caution:* Do not tap the culture vigorously as this may cause spills or excessive foaming of the culture, which may denature the proteins in the medium.

Pipettes: Use only sterile, disposable pipettes or glass pipettes sterilized in a canister. The practice of *pipetting by mouth has been discontinued* to eliminate the possibility of autoinfection by accidentally imbibing the culture or infectious body fluids. Instead, a mechanical pipetting device is to be used to obtain and deliver the material to be inoculated.

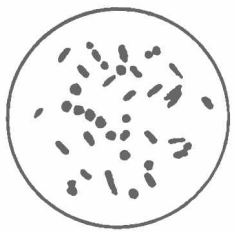
Incubation Procedure

Microorganisms exhibit a wide temperature range for growth. However, for most used in this manual, optimum growth occurs at 37°C over a period of 18 to 24 hours. Unless otherwise indicated in specific exercises, incubate all cultures under the conditions cited above. Place culture tubes in a rack for incubation. Petri dishes may be stacked; however, they *must always be incubated in an inverted position (top down)* to prevent water condensation from dropping onto the surface of the culture medium. This resultant excess moisture may then serve as a vehicle for the spread of the microorganisms on the surface of the culture medium, thereby producing confluent rather than discrete microbial growth.

Procedure for Recording Observations and Results

The accurate accumulation of experimental data is essential for the critical interpretation of the observations upon which the final results will be based. To achieve this end, it is imperative that you complete all the preparatory readings that are necessary for your understanding of the basic principles underlying each experiment. Meticulously record all the observed data in the "Observations and Results" section of each experiment.

In the exercises that require drawings to illustrate microbial morphology, it will be advantageous to depict shapes, arrangements, and cellular structures enlarged to 5 to 10 times their actual microscopic size, as indicated by the following illustrations. For this purpose a number 2 pencil is preferable. Stippling may be used to depict different aspects of cell structure (e.g., endospores or differences in staining density).



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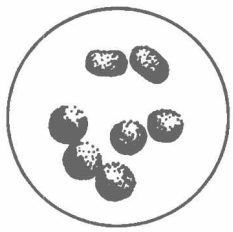

Good drawing

Review Questions

The review questions are designed to evaluate student's understanding of the principles and the interpretations of observations in each experiment. Completion of these questions will also serve to reinforce many of the concepts that are discussed in the lectures. At times, this will require the use of ancillary sources such as textbooks, microbiological reviews, or abstracts. The designated critical-thinking questions are designed to stimulate further refinement of cognitive skills.

Procedure for Termination of Laboratory Sessions

1. Return all equipment, supplies, and chemical reagents to their original locations.
2. Neatly place all capped test tube cultures and closed Petri dishes in a designated collection area in the laboratory for subsequent autoclaving.
3. Place contaminated materials, such as swabs, disposable pipettes, and paper towels, in a biohazard receptacle prior to autoclaving.
4. Carefully place hazardous biochemicals, such as potential carcinogens, into a sealed container and store in a fume hood prior to their disposal according to the institutional policy.
5. Wipe down table top with recommended disinfectant.
6. Wash hands before leaving the laboratory.

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Basic Laboratory Techniques for Isolation, Cultivation, and Cultural Characterization of Microorganisms

LEARNING OBJECTIVES

Once you have completed the experiments in this section, you should be familiar with

1. The types of laboratory equipment and culture media needed to develop and maintain pure cultures.
2. The concept of sterility and the procedures necessary for successful subculturing of microorganisms.
3. Streak-plate and spread-plate inoculation of microorganisms in a mixed microbial population for subsequent pure culture isolation.
4. Cultural and morphological characteristics of microorganisms grown in pure culture.

INTRODUCTION

Microorganisms are ubiquitous. They are found in soil, air, water, food, sewage, and on body surfaces. In short, every area of our environment is replete with them. The microbiologist separates these mixed populations into individual species for study. A culture containing a single unadulterated species of cells is called a **pure culture.** To isolate and study microorganisms in pure culture, the microbiologist requires basic laboratory apparatus and the application of specific techniques, as illustrated in Figure I.1.

Media

The survival and continued growth of microorganisms depend on an adequate supply of nutrients and a favorable growth environment. For the former, most microbes must use soluble low-molecular-weight substances that are frequently derived from the enzymatic degradation of complex nutrients. A solution containing these nutrients is a **culture medium.** Basically, all culture media are liquid, semisolid, or solid. A liquid medium lacks a solidifying agent and is called a **broth medium.** A broth medium supplemented with a solidifying agent called **agar** results in a solid or semisolid medium. Agar is an extract of seaweed, a complex carbohydrate composed mainly of galactose, and is without nutritional value. Agar serves as an excellent solidifying agent because it liquefies at 100°C and solidifies at 40°C. Because of these properties, organisms, especially pathogens, can be cultivated at temperatures of 37.5°C or slightly higher without fear of the medium liquefying. A completely solid medium requires an agar concentration of about 1.5 to 1.8%. A concentration of less than 1% agar results in a **semisolid medium.** A solid medium has the advantage that it presents a hardened surface on which microorganisms can be grown using specialized techniques for the isolation of discrete colonies. Each colony is a cluster of cells that originates from the multiplication of a single cell and represents the growth of a single species of microorganism. Such a defined and well-isolated colony is a **pure culture.** Also, while

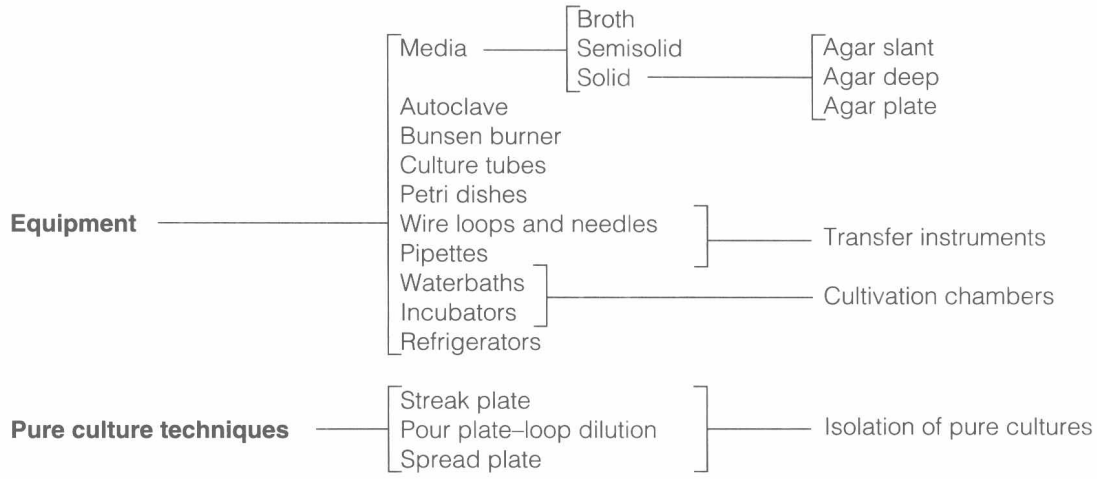

FIGURE I.1 Laboratory apparatus and culture techniques

in the liquefied state, solid media can be placed in test tubes, which are then allowed to cool and harden in a slanted position, producing **agar slants.** These are useful for maintaining pure cultures. Similar tubes that, following preparation, are allowed to harden in the upright position are designated as **agar deep tubes.** Agar deep tubes are used primarily for the study of the gaseous requirements of microorganisms. However, they may be liquefied in a boiling water bath and poured into Petri dishes, producing **agar plates,** which provide large surface areas for the isolation and study of microorganisms. The various forms of solid media are illustrated in Figure I.2.

In addition to nutritional needs, the environmental factors must also be regulated, including proper pH, temperature, gaseous requirements, and osmotic pressure. A more detailed explanation is presented in Part IV, which deals with cultivation of microorganisms; for now, you should simply bear in mind that numerous types of media are available.

Sterilization

Sterility is the hallmark of successful work in the microbiology laboratory. To achieve sterility, it is mandatory that you use sterile equipment and sterile techniques. **Sterilization** is the process of rendering a medium or material free of all forms of life. Although a more detailed discussion is presented in Part IX, which describes the control of microorganisms, Figure I.3 is a brief outline of the routine techniques used in the microbiology laboratory.

Culture Tubes and Petri Dishes

Glass **test tubes** and glass or plastic **Petri dishes** are used to cultivate microorganisms. A suitable nutrient medium in the form of broth or agar may be added to the tubes, while only a solid medium is used in Petri dishes. A sterile environment is maintained in culture tubes by various types of closures. Historically, the first type, a cotton plug, was developed by Schröeder and von Dusch in the nineteenth century. Today most laboratories use sleevelike caps (Morton closures) made of metal, such as stainless steel, or heat-resistant plastics. The advantage of these closures over the cotton plug is that they are labor-saving and, most of all, slip on and off the test tubes easily.

Petri dishes provide a larger surface area for growth and cultivation. They consist of a bottom dish portion that contains the medium and a larger top portion that serves as a loose cover. Petri dishes are manufactured in various sizes to meet different experimental requirements. For routine purposes, dishes approximately 15 cm in diameter are used. The sterile agar medium is dispensed to previously sterilized dishes from molten agar deep tubes containing 15 to 20 ml of medium, or from a molten sterile medium prepared in bulk and contained in 250-, 500-, and 1000-ml flasks, depending on the volume of medium required. When cooled to 40°C, the medium will solidify. Remember that *after inoculation, Petri dishes are incubated in an inverted position* (top down) to prevent condensation that forms on the cover during